

Influence of *Bacillus coagulans* on the Compressive Strength and Durability of Concrete

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Abstract

This study investigates the influence of *Bacillus coagulans* on the compressive strength and durability of concrete. Concrete cubes were prepared in three different ways. The first set was prepared without bacteria treatment as control. Second set was prepared by mixing concrete with *B. coagulans* suspension at various densities and then cured in water while the third set was prepared without the bacteria but cured in cementation reagent containing various *B. coagulans* suspension densities. 1.5×10^8 cells/ml, 12.0×10^8 cells/ml, and 24.0×10^8 cells/ml *B. coagulans* suspension densities of bacteria were investigated for bacteria-treated-concrete. The 100mm concrete cubes were tested for compressive strength and durability under Sulfuric acid environment. Tests were conducted between 7 and 28days at 7days interval. In general, concrete treated with bacteria were much stronger than the control at early ages up to 14days. Although they were still stronger at 28 days, the percentage increase in strength were much lower. At 28 days, the compressive strength of the untreated concrete was 32N/mm^2 . For concrete mixed with bacteria, the highest compressive strength was 34N/mm^2 obtained at 12.0×10^8 cells/ml bacteria density while 37N/mm^2 was obtained at the same density for concrete cured in bacteria suspension. The durability study indicated that concrete treated with bacteria performed better than the control. For both mixing and curing with bacteria, the best result of loss of strength at 28days were obtained at bacteria density of 1.5×10^8 cells/ml; 12.0% and 13.7% respectively compared to 16.5% for the control. Analysis of variance (ANOVA) test shows that concrete immersed in bacteria reagent has more significant effect on the compressive strength than those prepared by mixing the bacteria with the concrete. Microbial calcite precipitation was viewed using a scan electron microscope (SEM), which established the presence of calcite precipitation inside pores with bacterial impression and a new calcite layer on the surface of the concrete formed.

Keywords: *Bacillus coagulans*, Concrete, Compressive strength and Durability

1. Introduction

Concrete is apparently the most commonly used building material (normally used on its own or in combination with other materials) though attended with its peculiar drawbacks. In concrete, cracking is a common phenomenon due to its relatively low tensile strength and the prevalence of micro pores also reduces its durability. Therefore, when the material is used under sustained loading and in aggressive environments, the life of the structure may be reduced due to ingress of deteriorating agents. In view of these drawbacks and the desire to prolong the life of concrete structures, several methods and measures have been explored to improve the strength and quality of concrete. Of recent, microbiologically induced calcium carbonate precipitation (MICCP) resulting from metabolic activities of some specific microorganisms in concrete to improve the overall behaviour of concrete has begun to attract interest of researchers. It has been seen as an alternative and an environment friendly means for the improvement of strength and quality of building materials (Mayur, 2013).

The technique can be used to improve the compressive strength and stiffness of concrete. Often, bacterial activities simply trigger a change in solution of chemistry that leads to over saturation and mineral precipitation. Use of these bio-mineralogy concepts in concrete leads to potential invention of new material called 'Bacterial Concrete'. Research leading to microbial calcium carbonate precipitation and its ability to heal cracks of construction materials has led to many applications like crack remediation of concrete, sand consolidation, restoration of historical monuments and other such applications (Mayur, 2013). Integrated bacteria would thus represent an internal self-healing agent which autonomously decreases matrix permeability upon crack formation, save manual repair and moreover increase structural durability thereby saving both money and the environment as less maintenance and use of environmental unfriendly repair materials could be avoided. The technique is adopted in remediation of cracks and fissures in concrete by utilizing Microbiologically Induced Calcite or

Calcium Carbonate (CaCO_3) Precipitation (MICP) and it is a technique that comes under a broader category of science called bio-mineralization. MICP is highly desirable because the Calcite precipitation induced as a result of microbial activities is pollution-free and natural. Transport properties and mechanical properties (compressive strength) are the important factors for concrete durability.

Previous studies with aerobic microorganism such as *Bacillus pasteurii* and *Pseudomonas aeruginosa* showed a significant improvement (about 18%) in compressive strength of cement mortar (Ramakrishnan *et al.*, (1998); Ramakrishnan *et al.*, (2001). A recent study with the use of *Bacillus pumilus* also indicate an early gain in strength but recorded an increase in compressive strength of just 6.5% at 28days accompanied with better performance under sulphuric environment when compared to untreated concrete (Oriola *et al.*, (2018).

Since it is generally accepted that the durability of concrete is related to the characteristics of its pore structure as the deterioration process is dependent on movement of harmful agents into the concrete matrix through interconnected pores or cracks (Claisse *et al.*, 1997, Khan 2003), the MICP process continues to be of interest as the search for bacteria which will be most the effective in reducing the porosity or permeability of concrete continues. Though all bacteria are capable of CaCO_3 production, recent researches have shown that only some specific species of bacteria can be useful to enhance the durability and strength of concrete structures; those that can survive in the alkaline environment inside concrete (Senot, 2017). Therefore this study focuses on determining the effect of *B. coagulans* precipitate on the strength and durability of concrete. *B. coagulans* is a common soil bacterium which thrives in alkaline soil environment and is expected to survive in concrete and induce the precipitation of strong and durable calcite in concrete.

2.0 Materials And Methods

2.1 Materials

- Cement: Ordinary Portland Cement of 42.5 grade available in local market was used in the investigation.
- Fine Aggregate: Natural river sand having specific gravity of 2.60 was used.
- Coarse Aggregate: 20mm maximum aggregate size, crushed angular granite from local quarry was used as coarse aggregate in this investigation.
- Water: Tap water (potable) was used for mixing and curing (where appropriate) during the laboratory investigation.
- Bacillus coagulans*: The *B. coagulans* being urease-producing bacteria was used for this study and it is classified according to American Type Culture Collection as ATCC 7050. The role of bacteria (*B. coagulans*) is to produce enzyme urease through its metabolic activities under proper cultivation process. The urease enzyme catalyzes the MICP biochemical reaction of hydrolyzing urea. The suspension of various densities of *B. coagulans* are shown in Fig. 1.
- Cementation Reagent: Cementation reagents serve as raw materials for calcite formation in the MICP process. The cementation reagent that was employed in this study comprises a solution of 20g of urea ($\text{CO}(\text{NH}_2)_2$), 2.8g of calcium chloride (CaCl_2), 3g of nutrient broth, 10g of ammonium chloride (NH_4Cl), and 2.12g sodium bicarbonate (NaHCO_3) per litre of deionized water (DeJong *et al.*, 2006; Qabany *et al.*, 2011; Stocks-Fischer *et al.*, 1999; Stoner *et al.*, 2005).
- Sulfuric acid: Sulfuric acid was used to provide the acid environment required for the short time durability test. It comprises Ammonia (NH_3) -0.0005%, Arsenic (As) -0.00001%, Chloride (Cl) -0.0002%, Heavy metals (Pb)-0.0001%, Iron (Fe)-0.0001%, Nitrate (NO_3)-0.00002%, Oxygen absorbed (O)-0.00015%, Non-volatile residue-0.0025%, and weighs 1.84g/ml

2.2 Methods

2.2.1 Concrete Production

a) Mix design and casting

A prescribed mix, 0.55/1:2:3 targeting a concrete strength of Class C25/30 was adopted after some trial mix tests. 100mm cubes were adopted and in all, 96 cubes of concrete were cast; each test result represents an average of 3 cube results. There were 8 sets of 12 cubes; two sets were for control for compressive strength test and durability test. Three sets were for concrete mixed with various suspension density of *B. coagulans* and the other three sets were for concrete cured in various suspension density of *B. coagulans*. Mixing was by hand until a good consistency was achieved. Casting was done using a tampering rod in accordance with BS EN 12390-2:2000.; samples of fresh concrete cubes are as shown in Fig. 2. Cubes after casting were de-molded after one day and cured appropriately.

b) Control Specimen

The control samples were mixed with the appropriate amount of water only and demolded after 24hrs, thereafter they were cured in water at ambient temperature (about 30°C).

c) Concrete mixed with bacteria

The same mix for the control was adopted but 250ml of various suspension density (1.5×10^8 cells/ml, 12.0×10^8

cells/ml, and 24.0×10^8 cells/ml) of *B. coagulans* solution were used to replace equal volume of water of the water-cement ratio so as not to alter the workability of the concrete. The cast cubes were de-molded and cured in water at ambient temperature (about 30°C)

d) Concrete cured with bacteria

The concrete was mixed as per the control with water only, they were demolded after 24hrs and then cured in cementation reagent solution in which 250ml of various suspension density (1.5×10^8 cells/ml, 12.0×10^8 cells/ml, and 24.0×10^8 cells/ml) of *B. coagulans* were added into three separate curing tank which was used for the curing of the cubes.

2.2.2 Compressive Strength Test

The compressive strengths of the mixes were determined at ages 7, 14, 21 and 28 days according to the provisions of BS EN 12390-3:2001. The compressive test was conducted in the Civil Engineering laboratory of Nigeria Defence Academy, Kaduna. Fig. 3 shows the picture of the ADR 2000BS Analogue Type Compression Testing Machine used.

2.2.3 Durability Test (resistance against acid attack)

After 28 days curing (in water or bacteria solution), the remaining cubes were removed from the curing medium and allowed to dry in the laboratory for 24hrs and then immersed in 10% solution of H_2SO_4 . The cubes were removed in groups from the solution and tested after 7, 14, 21 and 28days of immersion. The response of the cubes to the solution was evaluated through change in appearance, weight, compressive strength and thickness that was observed. Fig. 4 shows the effect of the sulphuric acid on the concrete. The percentage weight lost is calculated thus:

$$\text{Weight loss \%} = \frac{\text{Loss in specimen weight}}{\text{Initial specimen weight}} \times 100$$

2.2.4 Scanning Electron Microscopy (SEM)

The deposition of calcite inside the micro cracks of concrete by bacteria was analyzed under scanning electron microscope (SEM). SEM is a powerful magnification tool that utilizes focused beams of electrons to obtain information. The high resolution, three dimensional images produced by SEMs provide topographical, morphological and compositional information thus making them invaluable in a variety of science and industrial applications.

3.0 Discussion Of Results

3.1 Compressive strength test for concrete mixed with *Bacillus coagulans*

The compressive strength tests results at various stepped densities of *B. coagulans* suspension for concrete mixed with bacteria is shown in Fig. 5. The compressive strength generally shows an increasing trend with increase in curing days. The general trend is presented in Table 1; the table shows that within the limits of experimental errors, the trend of the growth in strength is unaffected by the presence of bacteria, however, the strengths of bacteria-concrete were in general higher than that of the control except for the $1.5E8$ bacteria concentration. The improvement in strength above that of the control may be as a result of the calcite precipitate formed as *B. coagulans* produce enzyme urease through its metabolic activity and the enzyme urease triggered the MICP biochemical reaction by hydrolyzing urea ($CO(NH_2)_2$). The ammonium (NH_4^+) produced increased the pH and caused the bicarbonate (HCO_3^-) to precipitate with calcium ion (Ca^{2+}) from the calcium chloride supplied in order to form the calcium calcite ($CaCO_3$).

Table 2 shows the percentage differences in compressive strength of the bacteria concrete when compared to the control at different ages. Results as presented in Table 2 indicate that apart from the lowest suspension density of $1.5E8$ cells/ml *B. coagulans*, concrete mixes with bacteria addition performed better at early age than the control in terms of compressive strength. However, the compressive strengths at 28 days were not significantly different, the highest compressive strength increase was achieved with an addition of $12E8$ cells/ml of *B. coagulans* suspension and it was 6.25% above the compressive strength of the control. With an additive of *B. coagulans* suspension density of $1.5E8$ cells/ml, the compressive strength was lower than that of the control at age 14 and 21 days but the same at 28 days of curing; this implies that densities of *B. coagulans* suspension higher than $1.5E8$ cells/ml are required for beneficial effect on compressive strength.

A two-way analysis of variance (ANOVA) for the compressive strength test for concrete mixed with bacteria is summarized in Table 3. The results show that only the effect of curing days on the compressive strength was statistically significant ($F_{CAL} = 65.96 > F_{CRIT} = 3.863$) for curing days while effect of *B. coagulans* suspension density on the compressive strength was not statistically significant ($F_{CAL} = 3.60 < F_{CRIT} = 3.863$).

3.2 Compressive strength test for concrete cured in *Bacillus coagulans* suspensions

The compressive strength tests results at various stepped densities of *B. coagulans* suspension for concrete cured in bacteria is shown in Fig. 6. The compressive strength generally shows an increasing trend with increase in curing days for all densities. Table 4 shows the percentage strength development at different ages for different curing regime and density of bacteria.

Table 4 indicates that the strength development is within the range for normal concrete (Neville, 2011) but concrete cured with bacteria had significant early strength than the control (untreated concrete).

Furthermore, Fig. 6 indicates that concrete cured in solution of bacteria with a concentration of 1.5×10^8 and 12×10^8 cells/ml of *B. coagulans* suspension density performed generally better than the control at all ages but with density of 24×10^8 cells/ml, concrete compressive strength was higher only at 7 & 14 days. This is an indication that for compressive strength improvement, a bacteria density of 12×10^8 cells/ml will be adequate, this is further confirmed with the fact that the highest compressive strength (37 N/mm^2) at 28 days was achieved at this bacteria density. Table 5 also compares the performance of concrete cured in bacteria solution with that of the control at different ages.

Analysis of the results as shown Table 5 confirms the early strength gain above that of the control but the trend was decreasing towards the 28 days. This is an indication that bacteria was able to penetrate the concrete matrix in early ages when there was prevalence of interconnecting pores (Jagadeesha *et al.*, (2013)) but with increasing production of cement hydration products and calcite precipitate, pores were sealed up and therefore this reduced the effect of bacteria. It is also possible that as the pH of the specimen became high as curing period was increased, cells activity reduced gradually. The improvement in the compressive strength of concrete specimens based on MICP produced by *B. coagulans* is supported by previous studies (Ramakrishnan, *et al.*, 2005; Bang, *et al.*, 2010; Ghosh *et al.*, 2005 and Oriola *et al.*, 2018).

Irrespective of the type of bacteria concrete treatment adopted, it was found that concrete mixed or cured in 1.5×10^8 cells/ml and 12×10^8 cells/ml bacterial concentration performed better than 24×10^8 cells/ml. A two-way analysis of variance (ANOVA) for the compressive strength test for concrete cured in bacteria is summarized in Table 6. The results show that the effect of curing days and *B. coagulans* concentration on the compressive strength were statistically significant ($F_{\text{CAL}} = 16.64 > F_{\text{CRIT}} = 3.863$) for curing days and ($F_{\text{CAL}} = 5.15 > F_{\text{CRIT}} = 3.863$) for *B. coagulans* concentration. The effect of curing days was more pronounced than that of *B. coagulans* concentration.

The result from Table 2 and Table 5 shows that *B. coagulans* concentration has more effect on the compressive strength of concrete when the concrete is cured in cementation reagent containing various *B. coagulans* suspension density solution than when the various *B. coagulans* suspension density are used in mixing the concrete.

3.3 Durability test results for concrete mixed with bacteria

The variation of percentage loss in strength for concrete immersed in Sulphuric acid for concrete mixed with bacteria is as shown in Figure 7. The general trend is increasing loss in weight with the period of immersion but the rate of weight loss decreased with age of immersion. Concrete prepared with bacteria performed better than the control at all ages with the least concentration of 1.5×10^8 cells/ml of bacteria performing best; at 28 days of exposure to sulphuric environment, the weight loss for 1.5×10^8 *B. coagulans* cells/ml density was 12% compared to 16.5% for the control.

A two-way analysis of variance (ANOVA) for the durability test for concrete mixed with bacteria is summarized in Table 7. The results show that the effect of curing days and *B. coagulans* concentration on durability were statistically significant ($F_{\text{CAL}} = 265.75 > F_{\text{CRIT}} = 3.863$) for curing days and ($F_{\text{CAL}} = 65.06 > F_{\text{CRIT}} = 3.863$) for *B. coagulans* concentration. The effect of curing days was more pronounced than that of *B. coagulans* concentration.

3.4 Durability test results for concrete cured in cementation reagent containing various *Bacillus coagulans* suspension density

The variation of percentage loss in strength for concrete immersed in sulphuric acid environment for concrete cured in bacteria is shown in Figure 8. Again, the general trend of the weight loss is increase with age but at a decreasing rate. The result of this durability test on concrete cured in bacteria suspensions shows that lower percentage weight loss was achieved at bacteria concentration of 12×10^8 cells/ml for all the curing period. Although the values recorded for 1.5 and 12×10^8 cells/ml are very close and smaller than those recorded at 24×10^8 cells/ml of *B. coagulans* suspension density. The percentage weight loss recorded at 28 days were 16.5%, 13.7%, 14% and 16.6% for control, 1.5×10^8 bacteria cells/ml, 12×10^8 bacteria cells/ml and 24×10^8 bacteria cells/ml respectively.

A two-way analysis of variance (ANOVA) for the durability test for concrete cured with bacteria is summarized in Table 8. The results show that the effect of immersion days and *B. coagulans* concentration on durability were statistically significant ($F_{\text{CAL}} = 112.596 > F_{\text{CRIT}} = 3.863$) for curing days and ($F_{\text{CAL}} = 13.26 > F_{\text{CRIT}} = 3.863$) for *B. coagulans* concentration. The effect of curing days was more pronounced than that of *B. coagulans* concentration.

3.5 Scanning electron microscope (SEM)

The scanning electron microscope (SEM) examination was made on the control and MICP samples collected from the cubes tested at 28 days. No precipitate was observed in the control samples (see Fig. 8a) wherein, pores can be easily seen inside it. A clear calcite precipitation was found on the crack remediated area in the samples containing the bacteria cells (see Fig. 8b and c). The SEM analysis of concrete mixed or cured with *B. coagulans* has revealed distinct calcite crystals embedded in concrete. High calcium amounts in it confirmed that calcite was present in the form of calcium carbonate due to bacteria action. Fig. 8b shows the presence of crystalline calcium carbonate associated with bacteria. The deposition of calcite serves as barrier to reduce porosity and thus improves impermeability and strength of the concrete similar observations were made by (Chahal *et al.*, (2012) and Abo-El-Enein *et al.*, (2013) and Oriola *et al.*, (2018)).

4.0 Conclusion

Based on the results of this investigation the following conclusions are made:

1. *B. coagulans* treated concrete showed improved compressive strength at early age above that of untreated samples.
2. Highest compressive strength values were obtained at 1.5×10^8 cells/ml and 12.0×10^8 cells/ml for concrete cured in cementation reagent containing various *B. coagulans* suspension density and concrete mixed with bacteria respectively.
3. The SEM revealed that strength gain is as a result of the deposition of the calcite material by the bacterial activity.
4. The compressive strength and durability of *B. coagulans* treated concrete decreases with higher concentration of *B. coagulans*.
5. Concrete cured in bacteria solution performed better than those mixed with bacteria for compressive strength and durability.
6. The optimum *B. coagulans* cells suspension density which leads to the highest improvement of the compressive strength and durability of concrete treated with *B. coagulans* is 12.0×10^8 cells/ml

5.0 Recommendation

B. coagulans suspension density of 12.0×10^8 cells/ml is recommended for bacteria concrete and the concrete should be cast with water but cured in cementation reagent containing the *Bacillus coagulans*.

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Figure 1: Cultured Bacterial Samples



Figure 2: Fresh concrete cube



Figure 3: Compressive testing machine



Figure 4: The effect of Sulphuric acid on concrete

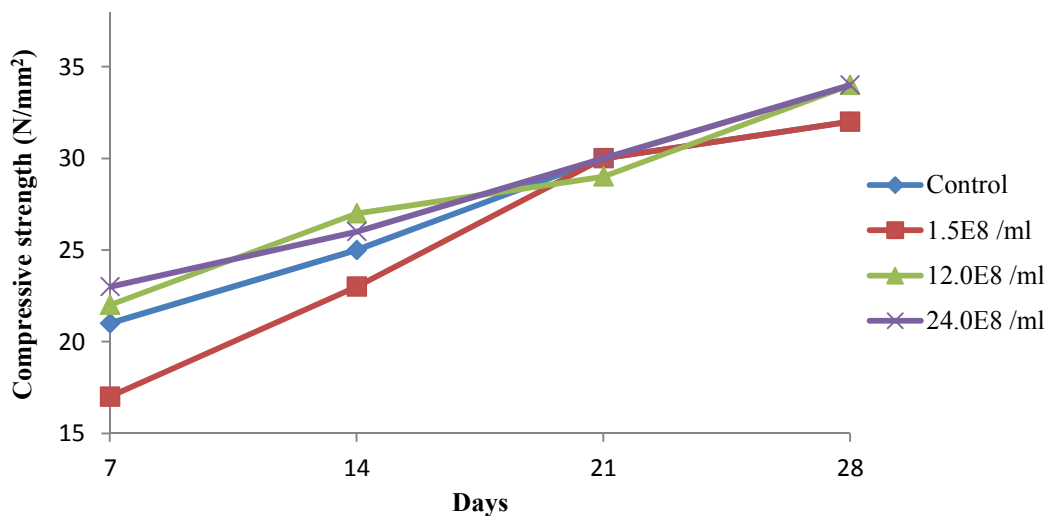


Figure 5: Comparison of compressive strength results of control and concrete made with various *Bacillus coagulans* suspension density

Table 1: Trend of Compressive Strength Development for various Densities of bacteria in mix: Percentage of Strength at Different Ages compared with 28days strength

Age Days	Concentration of Bacteria				Average
	0 (Control)	1.5E8 cells/ml	12E8 cells/ml	24E8 cells/ml	
7	66%	53%	65%	68%	63%
14	79%	72%	79%	77%	77%
21	94%	94%	85%	88%	90%
28	100%	100%	100%	100%	100%

Table 2: Percentage Difference Between the Compressive Strength of Bacteria-Mixed-Concrete Compared with that of Control at Different Ages

Age (Days)	Concentration of Bacteria			
	0 (Control)	1.5E8 cells/ml	12E8 cells/ml	24E8 cells/ml
7	0.00%	-19.0%	4.76%	9.52%
14	0.00%	-8.00%	8.00%	4.00%
21	0.00%	0.00%	-3.33%	0.00%
28	0.00%	0.00%	6.25%	6.25%

Table 3: Two-way analysis of variance results for Compressive strength test for concrete mixed with bacteria

ANOVA						
Source of Variation	Sum off Squares	df	MS	F _{CAL}	P-value	F _{CRIT}
Curing Days	342.19	3	114.06	65.96	1.90E-06	3.863
Bacteria Treatment	18.69	3	6.23	3.60	0.0588	3.863
Error	15.56	9	1.73			
Total	376.44	15				

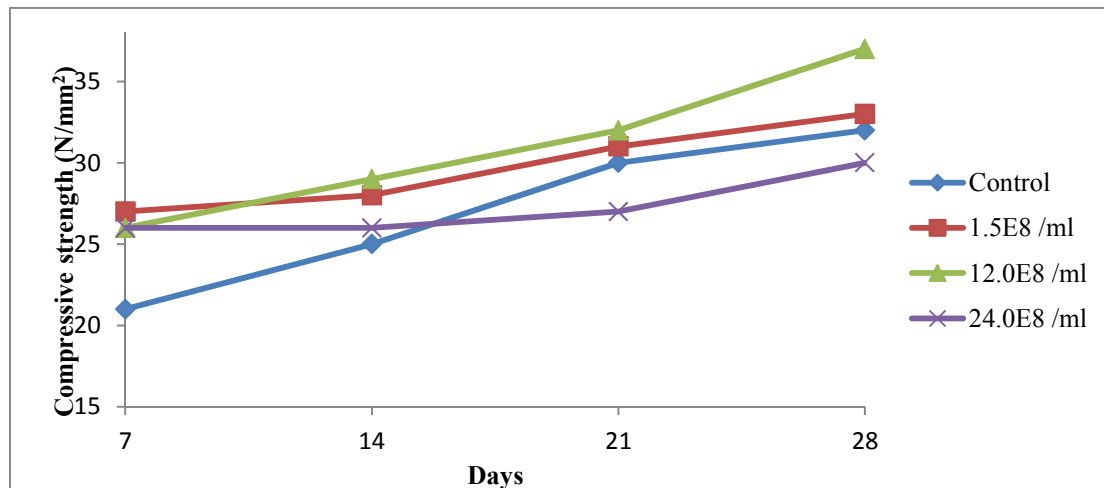


Figure 6: Comparison of compressive strength results of control and concrete immersed in cementation reagent containing various *Bacillus coagulans* suspension density

Table 4: Percentage of compressive strength at different ages for different densities of bacteria in curing medium.

Age Days	Density of Bacteria				Average
	0 (Control)	1.5E8 cells/ml	12E8 cells/ml	24E8 cells/ml	
7	66%	82%	70%	86%	76%
14	79%	85%	78%	87%	82%
21	94%	94%	86%	87%	90%
28	100%	100%	100%	100%	100%

Table 5: Comparison of the Compressive Strength of Bacteria Solution Cured Concrete and Concrete cured in water (Control) at Different Ages

Age (Days)	Concentration of Bacteria			
	0 (Control)	1.5E8/ml	12E8/ml	24E8/ml
7	0.0	28.6%	23.8%	23.8%
14	0.0	12.0%	16.0%	4.0%
21	0.0	3.3%	6.7%	-10.0%
28	0.0	3.1%	15.6%	-6.3%

Table 6: Two-way analysis of variance results for compressive strength test for concrete cured in *Bacillus coagulans* suspension of various densities.

ANOVA						
Source of Variation	Sum off Squares	df	MS	F _{CAL}	P-value	F _{CRIT}
Curing Days	147	3	49	16.64	5.14E-04	3.863
Bacteria Treatment	45.5	3	15.17	5.15	0.024	3.863
Error	26.5	9	2.94			
Total	219	15				

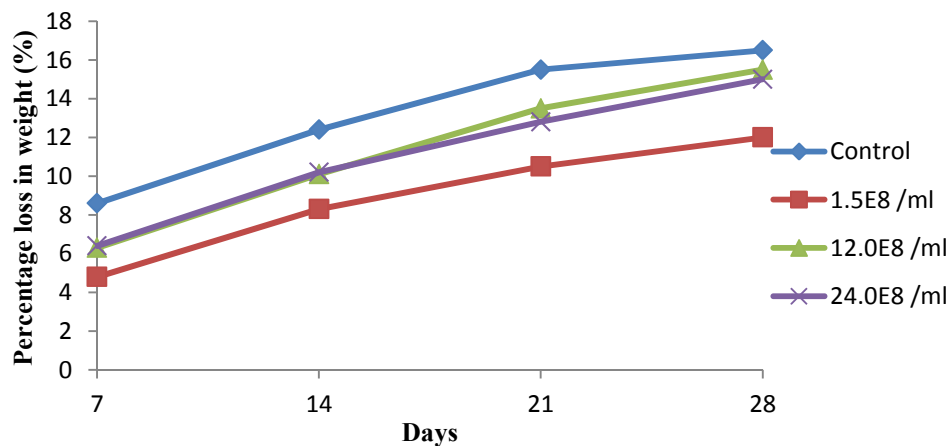


Figure 7: percentage weight loss comparison between control concrete and concrete mixed in various concentrations of bacteria

Table 7: Two-way analysis of variance results for durability test results for concrete mixed with bacteria

ANOVA						
Source of Variation	Sum off Squares	df	MS	F_{CAL}	P-value	F_{CRIT}
Curing Days	155.47	3	51.82	265.75	4.22E-09	3.863
Bacteria Density	38.06	3	12.69	65.06	2.01E-06	3.863
Error	1.76	9	0.196			
Total	195.28	15				

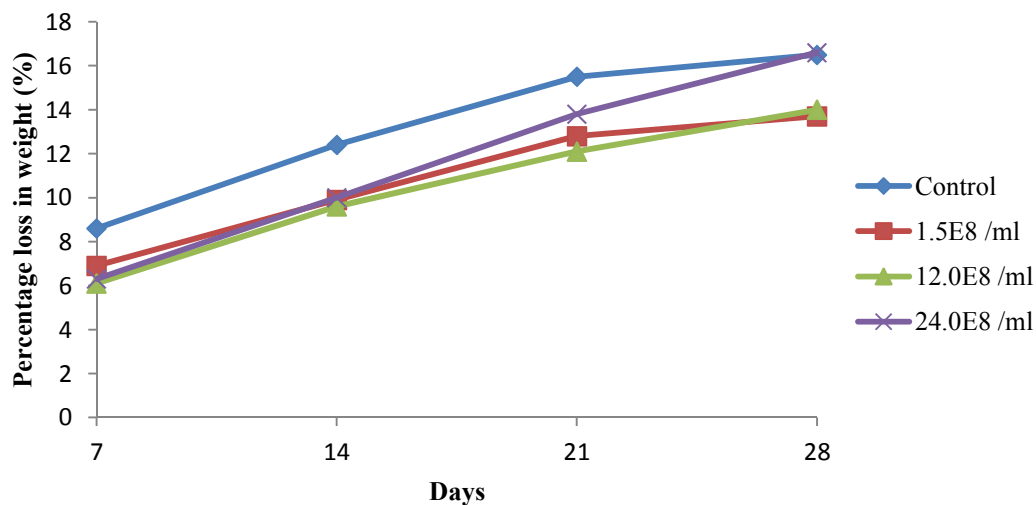


Figure 8: percentage weight loss comparison between control concrete and concrete immersed in cementation reagent containing various *Bacillus coagulans* suspension density

Table 8: Two-way analysis of variance results for durability test results for concrete immersed in cementation reagent containing various *Bacillus coagulans* suspension density

ANOVA						
Source of Variation	Sum off Squares	df	MS	F_{CAL}	P-value	F_{CRIT}
Immersion days	157.64	3	52.55	112.596	1.87E-07	3.863
Bacteria density	18.57	3	6.19	13.26	0.0012	3.863
Error	4.20	9	0.47			
Total	180.4	15				

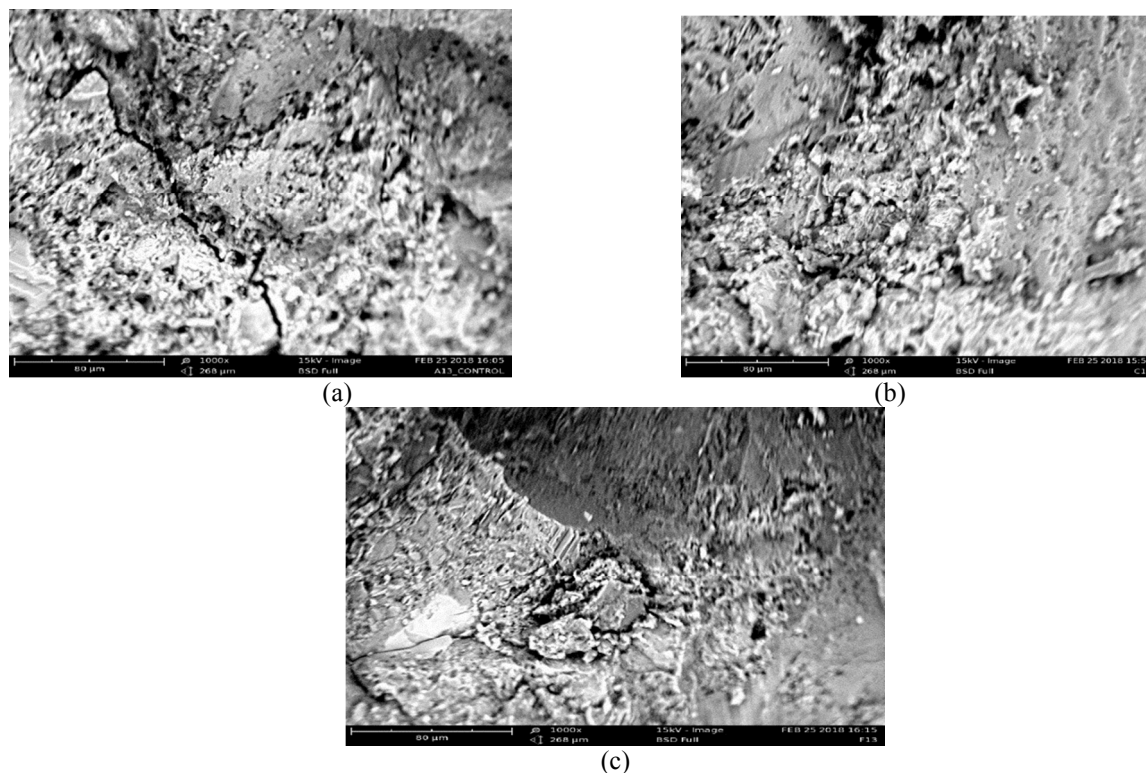


Figure 8: SEM images of (a) control concrete (b) concrete mixed with bacteria and (c) concrete cured in cementation reagent containing various *Bacillus coagulans* suspension density